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Research Article

Synthesis and Biological Evaluation of 1,3,4-Oxadiazolyl benzenesulphonyl benzimidazole derivatives

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Abstract

Oxadiazoles are a class of heterocyclic aromatic chemical compound of azole family. Oxadiazole is five membered heterocycle having two carbons, two nitrogen, one oxygen and two double bonds. Oxadiazole exists in four isomeric forms depending upon the position of nitrogen atom in the ring. Benzimidazole is a heterocyclic aromatic compound. This bicyclic compound consists of fusion of benzene and imidazole. Benzimidazole may also be considered as cyclic analogues of imidines due to tautomerism effect. In the present study involves synthesis of1,3,4-oxadiazolyl benzenesulphonylbenzimidazole derivatives. All the synthesized compounds were screened against HepG-2 cell line to determine the growth inhibitory effect of compounds.All the synthesized compounds possessed good to moderate anti-cancer activity as compare to standard drug Adriamycin. Two of the synthesized compounds *i.e.* **8a and 8f** were found to possess maximum anti-cancer activity.The structures of the synthesized compounds were established by IR and ^{1}HNMR.

Introduction

Cancer is a term given to diseases in which the cells divide abnormally and can invade other parts of body. One defining feature of cancer is the rapid creation of abnormal cells that grow beyond their usual boundaries, and which can then invade adjoining parts of the body and spread to other organs, the latter process is referred to as metastasizing.Cancer is considered to be the second most common cause of deaths after cardiovascular diseases in the world. Despite significantprogress has been achieved in anti-cancer therapy, the discoveryand development of effective anticancer drugs remain one ofthe most intractable worldwide health problems**[1]**. According to information from the World Health Organisation (WHO), it is estimated that there will be 12 million deaths from cancer in 2030. Numbers of clinically used compounds such as vincaalkaloids, colchicines, paclitaxel, and epothilone as anti-cancer agents attack microtubules by interfering with thedynamics of the tubulin polymerization and depolymerization, resulting in mitotic arrest[**2-5**].Undoubtedly, targeting tubulin is a successful strategy for cancer chemotherapy, however, thereare still many problems existing in clinical use of these anti-tubulin agents, like toxicity, poorwater solubility, poor bioavailability and multi-drug-resistant (MDR)**[6-8**]. Oxadiazoles are a class of heterocyclic aromatic chemical compound of azole family. Oxadiazole is five membered heterocycle having two carbons, two nitrogen, one oxygen and two double bonds **[9]**. Oxadiazole moiety and its various derivatives studied frequently in the past few decades and found potent in various pharmacological and pathological conditions. Due to its wide range of activities *vi*zanti-cancer **[10]**, anti-bacterial **[11]**, anti-fungal **[12]**, anti-tubercular **[13]**, anti-convulscant**[14]**, anti-inflammatory **[15]** a steady research is going on in oxadiazole nucleus**.**1,2,5-oxadiazole, 1,2,4-oxadiazole, 1,3,4-oxadiazole are known but 1,2,3-oxadiazole is unstable ring open to form diazoketone tautomer. 1,3,4-oxadiazoles is a thermally stable molecule. The 1,3,4-oxadiazole undergoes number of reactions including electrophillic substitution, nucleophilic substitution, thermal and photochemical**[16].**1,3,4-oxadiazole have been extensively studied for the identification of potential anticancer agents. Recent studies have indicated that 1,3,4-oxadiazole derivatives exhibit potent anti-cancer activity against different cancer cell lines through the inhibition of different growth factors, enzymes and kinases including telomerase, histone deacetylase (HDAC), methionine aminopeptidase (MetAP), thymidylate synthase (TS), glycogen synthase kinase-3 (GSK), epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), and focal adhesion kinase (FAK)**[17]**. The SAR studies revealed that the antitumor activity is dependent on the nature of the substituents at position 2 of 1,3,4-oxadiazoles **[18].**Most active 1,3,4-oxadiazoles have been seen to inhibit tubulin

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polymerization or destabalise the microtubule system. For example **Zibotentan**is a marketed compound based upon 1,3,4-oxadiazole and possess excellent anti-cancer activity. It is an endothelial receptor antagonist and used for treatment of prostate cancer **[19]**.Benzimidazole is a heterocyclic aromatic compound. This bicyclic compound consists of fusion of benzene and imidazole. Benzimidazole may also be considered as cyclic analogues of imidines due to tautomerism effect. Benzimidazole acts as phamacophore ring to synthesize biologically active moieties **[20].**Benzimidazole and its derivatives exhibit various pharmacological activities such as anti-cancer **[21]**, anti-HIV **[22]**, anti-inflammatory **[23]**, anti-microbial **[24],** making it an indispensable anchor for the development of various new therapeutic agents. The most commonly used approach to synthesize benzimidazole includes condensation of arylenediamine with a carbonyl equivalent. For example **Bendamustine** is a marketed and potent anti-cancer agent based on benzimidazole nucleus. It functions as an alkylating agent and causes both inter and intra-strand cross-links between DNA bases resulting in cell death **[25]**.

Experimental

The commercial chemicals employed for the present work were purchased from Spectrochem, Sigma-Aldrich, Merck India and Loba Chem. All the solvents were used after distillation. Thin layer chromatography was performed on E Merck silica gel $60F_{254}$precoated plates and the identification was done under UV light. Various solvent systems used for developing chromatograms were ethyl acetate: *n*-hexane: methanol, *n*-hexane: ethyl acetate, benzene: acetone and ethyl acetate: hexane. Melting points were determined using melting point apparatus (Perfit India) by capillary method and are uncorrected. The identification and characterization of the compounds were carried out by determining melting point, IR and ^{1}HNMR. All the infra red (IR) spectra were recorded on Bruker α-E FTIR-ATR. ^{1}H NMR was recorded on Bruker Avance 2400 (400MHz) spectrometer using DMSO and $CDCl_3$as solvent at SAIF, Punjab University, Chandigarh and JEOL JNM-ECS400 (400MHz) spectrometer at Indian Institute of Technology (IIT).

Synthesis of benzimidazole (2a)

The mixture of *o*-phenylenediamine (5.43g, 0.04mol) and formic acid (3ml, 0.06mol) was heated on a water bath for 2 hours at 100°C. Cooled and then added 10% of sodium hydroxide solution until the mixture is just alkaline to litmus. Then above solution was filtered and washed with cold water twice. Then dissolved in boiling water and then added charcoal (1g) and then filtered hot, pure crystals were obtained.

Benzimidazole (2a): State: Colourless crystals; Yield: 75%; Melting point: 170-172°C; R_f 0.25 (Ethyl acetate: *n*-hexane: methanol, 3:2:1);IR (ν, cm^{-1}):1652 (C=N), 3107 (Ar-C-H), 1588 (Ar-C=C), 3107 (N-H); ^{1}HNMR ($CDCl_3$) 7.1-7.9 (m, 4H, Ar-H), 11.7 (s, 1H, NH).

Synthesis of 2-methyl benzimidazole (2b)

Heat the mixture of *o*-phenylenediamine dihydrochloride (5.42g, 0.03mol) and acetic acid (5.4ml, 0.09mol) and water (20 ml) under reflux for 45 minutes. Then the reaction was cooled and then basify by gradual addition of concentrated ammonia solution. Precipitates formed were recrystallized from 10% ethanol.

2-methyl benzimidazole (2b): State: Beige crystalline powder; Yield: 78%; Melting point: 176°C; R_f 0.46 (Ethyl acetate: *n*-hexane: methanol, 3:2:1);IR (ν, cm^{-1}):1618(C=N), 3047 (Ar-C-H), 1547 (Ar-C=C), 2978 (N-H); ^{1}HNMR($CDCl_3$) 2.51 (s, 3H, CH_3), 7.11-7.14 (m, 2H, Ar-H), 7.46-7.49 (m, 2H, Ar-H).

Synthesis of ester derivatives (4a-4e)

4-substituted benzoic acid (3a-3e) (8g)was dissolved in ethanol or methanol (20ml, 0.4mol) and then concentrated sulphuric acid (3ml, 0.03mol) was added which acts as catalyst. Then the above mixture was refluxed for 3-4 hours. Then the solution was cooled to room temperature. Precipitated formed were filtered out and recrystallized from ethanol to obtain ester derivatives (4a-4e).

4-Nitrobenzoic acid ethyl ester (4a): State: Colourless crystals; Yield: 82%; Melting point: 54-55°C; R_f 0.32 (Ethyl acetate: hexane 3:1);IR (ν, cm^{-1}):3114(Ar-C-H), 1710 (C=O), 1093 (C-O), 1600 (Ar-C=C), 1343 (Ar-NO_2); ^{1}HNMR (DMSO-d6) 8.26-8.45 (m, 2H, Ar-H), 8.26-8.12 (m, 2H, Ar-H), 4.44 (q, *J*=7.2 Hz, 2H, CH_2), 1.44 (t, *J*=7.1 Hz, 3H, CH_3).

4-Chlorobenzoic acid methyl ester (4b): State: White crystals; Yield: 80%; Melting point: 40-42°C; R_f 0.39 (Ethyl acetate: hexane 3:1);IR (ν, cm^{-1}):3135 (Ar-C-H), 1726 (C=O), 1113 (C-O), 1597 (Ar-C=C), 760 (Ar-Cl); ^{1}HNMR (DMSO-d6) 7.94 (d, *J*=8.6 Hz, 2H, Ar-H), 7.37 (d, *J*=8.6 Hz, 2H, Ar-H), 3.87 (s, 3H, CH_3).

4-Fluorobenzoic acid ethyl ester (4c): State: Yellow powder; Yield: 78%; Melting point: 25-27°C; R_f 0.65 (Ethyl acetate: hexane 3:1);IR (ν, cm^{-1}):3104(Ar-C-H), 1723 (C=O), 1083 (C-O), 1580 (Ar-C=C), 1350 (Ar-F); ^{1}HNMR (DMSO-d6) 8.04-8.09 (m, 2H, Ar-H), 7.08-7.14 (m, 2H, Ar-H), 4.38 (q, *J*=7.0, 2H, CH_2), 1.40 (t, *J*=7.0 Hz, 3H, CH_3).

4-Bromobenzoic acid methyl ester (4d): State: White powder; Yield: 76%; Melting point: 77-80°C; R_f 0.32 (Ethyl acetate: hexane 3:1);IR (ν, cm^{-1}):3130 (Ar-C-H), 1721 (C=O), 1110 (C-O), 1592 (Ar-C=C), 660 (Ar-Br); ^{1}HNMR (DMSO-d6) 7.87 (d, *J*=8.6 Hz, 2H, Ar-H), 7.55 (d, *J*=8.6 Hz, 2H, Ar-H), 3.87 (s, 3H, CH_3).

4-hydroxybenzoic acid ethyl ester (4e): State: White crystalline powder; Yield: 85%; Melting point: 115-116°C; R_f 0.24 (Ethyl acetate: hexane 3:1);IR (ν, cm^{-1}):3105 (Ar-C-H), 1730 (C=O), 1082 (C-O), 1578 (Ar-C=C), 3200 (Ar-OH); ^{1}HNMR (DMSO-d6) 7.87 (d, *J*=8.9 Hz, 2H, Ar-H), 6.81 (d, *J*=8.9 Hz, 2H, Ar-H), 4.3 (q, *J*=7.1 Hz, 2H, CH_2), 1.36 (t, *J*=7.1 Hz, 3H, CH_3), 5.0 (s, 1H,OH).

Synthesis of Hydrazide derivatives (5a-5e)

Mixture of compound (4a-4b) (9.5g) and ethanol (30ml, 0.6mol) was added to hydrazine hydrate (11ml, 0.2mol) and

the reaction mixture was refluxed for 3 hours. After cooling the solid precipitated was filtered, washed with ethanol, dried and recrystallized from ethanol to obtain desired derivatives (5a-5e).

4-Nitrobenzoic acid hydrazide (5a): State: Yellow powder; Yield: 75%; Melting point: 210-213°C; R_f 0.54 (n-hexane: ethyl acetate 3:1);IR (ν, cm^{-1}):1640 (C=O), 2850 (Ar- C-H), 1540 (Ar-C=C), 3300 (N-H), 1596 (N-H), 1340 (Ar-NO_2); ^{1}HNMR (DMSO-d6) 7.29-7.86 (m, 4H, Ar-H), 2.0 (s, 1H, NH), 4.13-4.14 (d, *J*=4, 2H, NH_2).

4-Chlorobenzoic acid hydrazide (5b): State: White crystals; Yield: 72%; Melting point: 260-262°C; R_f 0.56 (n-hexane: ethyl acetate 3:1);IR (ν, cm^{-1}):1655 (C=O), 2853(Ar-C-H), 1472 (Ar-C=C), 3304 (N-H), 1598 (N-H), 716 (C-Cl); ^{1}HNMR (DMSO-d6) 7.45-7.89 (m, 4H, Ar-H), 2.0 (s, 1H, NH), 8.0 (s, 2H, NH_2).

4-Fluorobenzoic acid hydrazide (5c): State: White powder; Yield: 76%; Melting point: 162-164°C; R_f 0.52 (n-hexane: ethyl acetate 3:1);IR (ν, cm^{-1}):1593 (C=O), 2864 (Ar-C-H), 1522 (Ar-C=C), 3049 (N-H), 1543 (N-H), 1342 (Ar-F).

4-Bromobenzoic acid hydrazide (5d): State: Light beige powder; Yield: 78%; Melting point: 167-169°C; R_f 0.62 (n-hexane: ethyl acetate 3:1);IR (ν, cm^{-1}):1533 (C=O), 2866 (Ar-C-H), 1519 (Ar-C=C), 3051(N-H), 1549 (N-H), 662 (Ar-F).

4-Hydroxybenzoic acid hydrazide (5e): State: Beige crystals; Yield: 74%; Melting point: 264-266°C; R_f 0.51 (n-hexane: ethyl acetate 3:1);IR (ν, cm^{-1}):1538 (C=O), 2861 (Ar- C-H), 1523 (Ar-C=C), 3054 (N-H), 1546 (N-H), 3190 (Ar-OH).

Synthesis of 2-(4-substituted)-5-phenyl-[1,3,4]oxadiazole derivatives (6a-6e)

Benzoic acid (3.5g, 0.02mol) was mixed with hydrazide derivative (5g) (5a-5e) and then 25 ml of phosphorus oxychloride (22ml, 0.1mol) was added on crushed ice. Then the above mixture was refluxed on heating mantle at low temperature for 2-3 hours. Then after completion of the reaction, the above solution was cooled to room temperature and then poured on ice. The precipitates formed were filtered, dried and recrystallised from ethanol.

2-(4-nitro-phenyl)-5-phenyl-[1,3,4]oxadiazole (6a): State: Off-white solid; Yield: 88%; Melting point: 144-146°C; R_f 0.62 (Benzene: acetone 7:3);IR (ν, cm^{-1}):3145 (Ar-C-H), 1478 (Ar-C=C), 1016 (C-O-C), 1590 (C=N), 1542 (Ar-NO_2); ^{1}HNMR ($CDCl_3$) 7.61-7.64 (t, *J*=6 Hz, 4H, Ar-H), 8.12-8.15 (t, *J*= 12Hz, 5H, Ar-H).

2-(4-chloro-phenyl)-5-phenyl-[1,3,4]oxadiazole (6b): State: Off-white powder; Yield: 82%; Melting point: 150-152°C; R_f 0.54 (Benzene: acetone 7:3);IR (ν, cm^{-1}):3134 (Ar-C-H), 1489 (Ar-C=C), 1009 (C-O-C), 1598 (C=N), 783 (Ar-Cl); ^{1}HNMR ($CDCl_3$) 7.49-7.53 (m, 5H, Ar-H), 8.06 (d, *J*= 8.0 Hz, 2H, Ar-H), 8.11 (d, *J*=6.6 Hz, 2H, Ar-H).

2-(4-fluoro-phenyl)-5-phenyl-[1,3,4]oxadiazole (6c): State: Off-white solid; Yield: 85%; Melting point: 138-140°C; R_f 0.76 (Benzene: acetone 7:3);IR (ν, cm^{-1}):3052 (Ar-C-H), 1472 (Ar-C=C), 1003 (C-O-C), 1545 (C=N), 1399 (Ar-Cl); ^{1}HNMR ($CDCl_3$) 7.22-727 (m, 2H, Ar-H), 7.54-7.57 (m, 3H, Ar-H), 8.13-8.18 (m, 4H, Ar-H).

2-(4-bromo-phenyl)-5-phenyl-[1,3,4]oxadiazole (6d): State: Light-brown solid; Yield: 87%; Melting point: 155-157°C; R_f 0.65 (Benzene: acetone 7:3);IR (ν, cm^{-1}):3267 (Ar-C-H), 1467 (Ar-C=C), 1018 (C-O-C), 1560 (C=N), 664 (Ar-Br).

4-(5-phenyl-[1,3,4]oxadiazole-2-yl)-phenol (6e): State: Off-white solid; Yield: 81%; Melting point: 170-172°C; R_f 0.69 (Benzene: acetone 7:3);IR (ν, cm^{-1}):3167 (Ar-C-H), 1457 (Ar-C=C), 1012 (C-O-C), 1550 (C=N), 3190 (Ar-OH); ^{1}HNMR ($CDCl_3$) 7.55-7.69 (m, 4H, Ar-H), 8.10-8.18 (m, 5H, Ar-H), 11.08 (s, 1H, OH).

Synthesis of 1,3,4-oxadiazolyl benzene sulfonyl benzimidazole derivative (8a-8j)

Substituted 1,3,4-oxadiazole derivative (6a-6e) (0.01mol) was mixed with chlorosulphonic acid (0.01mol) in equimolar proportions. This mixture was stirred for 30 minutes using mechanical stirrer. After stirring, the mixture was heated on boiling water bath for 2 hours. After completion of reaction, the solution was cooled to room temperature and then poured on ice. Precipitates formed were filtered and dried to obtain the desired derivatives (7a-7e).Firstly 4-(5-phenyl-[1,3,4]oxadiazole-2-yl)-benzenesulphonyl derivative (7a-7e) (0.05mol)was mixed with benzimidazole (2a) (0.59g, 0.005mol) and then dichloromethane (30ml, 0.3mol) and pyridine (1ml, 0.01mol) was added to above mixture. The above solution was stirred for 2-3 hours using mechanical stirrer. After completion of reaction, excess of solvent was removed by heating. After cooling, the precipitates formed were filtered out. Excess of pyridine was removed by using petroleum ether. The same procedure was repeated for reaction of 2-methylbenzimidazole (2b) and 4-(5-phenyl-[1,3,4]oxadiazole-2-yl)-bensulphonyl derivative (7a-7e). Final precipitates were recrystallised using ethanol to obtain the final derivatives.

2.7.1 1-{4-[5-(4-nitro-phenyl)-[1,3,4]oxadiazole-2-yl]-benzenesulphonyl}-1*H*-benzimidazole (8a):State: Off-white powder; Yield: 77%; Melting point: 251-252°C; R_f 0.56 (Ethyl acetate: hexane 3:1);IR (ν, cm^{-1}):1507 (Ar-C=C), 3096 (Ar-C-H), 1058 (S=O), 1602 (C=N), 1157 (C-O-C), 1330 (Ar-NO_2); ^{1}HNMR (DMSO) 8.35-8.41 (m, 4H, Ar-H), 8.1 (s, 2H, Ar-H), 7.63-7.65 (t, *J*=4 Hz, 4H, Ar-H), 7.2-73 (d, *J*=9 Hz, 2H, Ar-H), 8.6 (s, 1H, CH).

1-{4-[5-(4-chloro-phenyl)-[1,3,4]oxadiazole-2-yl]-benzenesulphonyl}-1*H*-benzimidazole (8b): State: Light-brown powder; Yield: 76%; Melting point: 254-259°C; R_f 0.67 (Ethyl acetate: hexane 3:1);IR (ν, cm^{-1}):1544 (Ar-C=C), 3057 (Ar-C-H), 1068 (S=O), 1599 (C=N), 1002 (C-O-C), 736 (Ar-Cl); ^{1}HNMR (DMSO) 7.60-7.68 (m, 8H, Ar-H), 8.5 (s, 1H, CH), 8.10-8.12 (t, *J*=4.4 Hz, 2H, Ar-H), 7.26 (s, 2H, Ar-H).

1-{4-[5-(4-fluoro-phenyl)-[1,3,4]oxadiazole-2-yl]-benzenesulphonyl}-1*H*-benzimidazole (8c):State: Beige powder; Yield: 79%; Melting point: 260-262°C; R_f 0.62 (Ethyl acetate: hexane 3:1);IR (ν, cm^{-1}):1544 (Ar-C=C), 2999 (Ar-C-

H), 1062 (S=O), 1675 (C=N), 999 (C-O-C), 1233 (Ar-F); ^{1}HNMR (DMSO) 7.28-7.65 (m, 8H, Ar-H) 8.16 (s, 2H, Ar-H), 8.08-8.10 (d, *J*=8 Hz, 2H, Ar-H), 8.68 (s, 1H, CH).

1-{4-[5-(4-bromo-phenyl)-[1,3,4]oxadiazole-2-yl]-benzenesulphonyl}-1*H*-benzimidazole (8d):State: Off-white powder; Yield: 78%; Melting point: 265-267°C; R_f 0.63 (Ethyl acetate: hexane 3:1);IR (ν, cm^{-1}):1590 (Ar-C=C), 2973 (Ar-C-H), 1063 (S=O), 1590 (C=N), 999 (C-O-C), 607 (Ar-Br); ^{1}HNMR (DMSO) 7.23-7.62 (m, 12H, Ar-H), 8.56 (s, 1H, CH).

4-{5-[4-(benzimidazol-1-sulphonyl)-phenyl]-[1,3,4]oxadiazole-2-yl}-phenol (8e): State: Off-white semi-solid; Yield: 73%; Melting point: 256-258°C; R_f 0.65 (Ethyl acetate: hexane 3:1);IR (ν, cm^{-1}):1550 (Ar-C=C), 3065 (Ar-C-H), 1070 (S=O), 1606 (C=N), 1011 (C-O-C), 2763 (Ar-OH); ^{1}HNMR (DMSO) 7.29-8.27 (m, 12H, Ar-H), 8.6 (bs, 1H, CH), 9.2 (s, 1H, OH).

1-{4-[5-(4-nitro-phenyl)-[1,3,4]oxadiazole-2-yl]-benzenesulphonyl}-2-methyl-1*H*-benzimidazole (8f):State: Beige powder; Yield: 75%; Melting point: 240-242°C; R_f 0.52 (Ethyl acetate: hexane 3:1);IR (ν, cm^{-1}):1507 (Ar-C=C), 3096 (Ar-C-H), 1058 (S=O), 1602 (C=N), 1247 (C-O-C), 1330 (Ar-NO_2),1331 (CH_3); ^{1}HNMR (DMSO) 7.59-7.65 (m, 5H, Ar-H), 8.12-8.14 (d, *J*=6 Hz, 2H, Ar-H), 8.37-8.43 (m, 5H, Ar-H), 2.45-2.46 (t, *J*=1.8 Hz, 3H, CH_3).

1-{4-[5-(4-chloro-phenyl)-[1,3,4]oxadiazole-2-yl]-benzenesulphonyl}-2-methyl-1*H*-benzimidazole (8g):State: Beige coloured powder; Yield: 72%; Melting point: 234-236°C; R_f 0.59 (Ethyl acetate: hexane 3:1);IR (ν, cm^{-1}): 1583 (Ar-C=C), 3057 (Ar-C-H), 1076 (S=O), 1628 (C=N), 1220 (C-O-C), 747 (Ar-Cl), 1405 (CH_3); ^{1}HNMR (DMSO) 8.54-8.55 (d, *J*=4.4 Hz, 2H, Ar-H), 7.59-7.68 (m, 8H, Ar-H), 8.1 (s, 2H, Ar-H), 2.69 (s, 3H, CH_3).

1-{4-[5-(4-fluoro-phenyl)-[1,3,4]oxadiazole-2-yl]-benzenesulphonyl}-2-methyl-1*H*-benzimidazole (8h):State: Off-white powder; Yield: 69%; Melting point: 221-223°C; R_f 0.57 (Ethyl acetate: hexane 3:1);IR (ν, cm^{-1}):1544 (Ar-C=C), 3062 (Ar-C-H), 1068 (S=O), 1603 (C=N), 1225 (C-O-C), 1298 (Ar-F), 1412 (CH_3); ^{1}HNMR (DMSO)7.42-8.16 (m, 12H, Ar-H), 2.7 (s, 3H, CH_3).

1-{4-[5-(4-bromo-phenyl)-[1,3,4]oxadiazole-2-yl]-benzenesulphonyl}-2-methyl-1*H*-benzimidazole (8i):State: Reddish powder; Yield: 70%; Melting point: 251-254°C; R_f 0.61 (Ethyl acetate: hexane 3:1);IR (ν, cm^{-1}):1586 (Ar-C=C), 2720 (Ar-C-H), 1063 (S=O), 1674 (C=N), 1216 (C-O-C), 608 (Ar-Br), 1393 (CH_3); ^{1}HNMR (DMSO) 7.28-7.60 (m, 12H, Ar-H), 2.62 (s, 3H, CH_3).

4-{5-[4-(2-methyl-benzimidazol-1-sulphonyl)-phenyl]-[1,3,4]oxadiazole-2-yl}-phenol (8j): State: Brown semi-solid; Yield: 71%; Melting point: 234-236°C; R_f 0.69 (Ethyl acetate: hexane 3:1);IR (ν, cm^{-1}):1548 (Ar-C=C), 2746 (Ar-C-H), 1070 (S=O), 1606 (C=N), 1214 (C-O-C), 3060 (Ar-OH), 1412 (CH_3); ^{1}HNMR (DMSO) 7.1-8.2 (m, 12H, Ar-H), 2.8 (s, 3H, CH_3), 8.6 (s, 1H, OH).

Anticancer Evaluation

***In-vitro* anti-cancer screening**

The anticancer screening of all the synthesized compounds was conducted against human lung cancer cells line (HepG-2) to determine the growth inhibitory effects of the compounds. Source of cell line was NCI, USA. Vehicle used for testing was Dimethylsulfoxide (DMSO). *In vitro* testing was done using SRB assay protocol; each derivative was tested at 4 dose levels (10 µg/ml, 20 µg/ml, 40 µg/ml, 80 µg/ml).

Using the seven absorbance measurements [time zero (Tz), control growth (C), and test growth in the presence of drug at the five concentration levels (Ti)], the percentage growth is calculated at each of the drug concentrations levels. Percentage growth inhibition is calculated as:

[(Ti-Tz)/(C-Tz)] x 100 for concentrations for which Ti>/=Tz

[(Ti-Tz)/Tz] x 100 for concentrations for which Ti<Tz.

Three dose response parameters GI_{50}, total growth inhibition (TGI) and LC_{50}were calculated for every compound.

Results and Discussion

As per the proposed protocol the synthesis of 1,3,4-oxadiazolyl benzenesulphonylbenzimidazole derivatives was carried out**.** The yield (%) of the said derivatives was found to be in the range of 50-84. The melting point of compounds ranged from 234-267˚C and are uncorrected. The $R_f$were observed in ranges of 0.52-0.69 using different solvents and detecting system**(Table 1)**. IR spectrum of benzimidazole showed C=N stretch at 1400-1650 cm^{-1}aromatic C-H stretch at 3000-3200 cm^{-1}and a broad N-H stretch at 2800-3300 cm^{-1}. The benzoic acid ester derivatives indicated characteristic C=O stretch at 1700-1725cm^{-1}, aromatic C-H stretch at 2970 cm^{-1}. IR spectrum of oxadiazole derivatives showed an absorption at 1615 cm^{-1}which confirmed the presence of (C=Nstr), an absorption at 1218 cm^{-1}was due to presence of (C-O-Cstr), an absorption at 3048 cm^{-1} was due to presence of (C-Hstr). IR spectrum of final compound showed an additional absorption at 1050 cm^{-1}due to presence of sulphoxide group. Solvent used for ^{1}HNMR spectrum was DMSO. The ^{1}HNMR spectrum of 1,3,4-oxadiazolyl benzenesulphonyl benzimidazole derivatives in general showed multiplet in the range of δ 8.1-8.4 for 4 aromatic protons of benzene ring of benzimidazole, another multiplet in the range of δ 7.2-7.6 for 4 aromatic protons of benzene ring attached to oxadiazole, a singlet at δ 8.6 confirmed the presence of N=CH and also a singlet in the range of 2.4-2.7 due 3 protons of CH_3.

Anticancer Drug Screening

All the synthesized compounds were screened against human lung cancer cell line (HepG-2) Sto determine the growth inhibitory effect of compounds. *In vitro* testing was done using SRB assay protocol, each derivative was tested at 4 dose levels (10 µg/ml, 20 µg/ml, 40 µg/ml, 80 µg/ml).

The synthesized1,3,4-oxadiazolyl benzenesulfonyl benzimidazole derivatives exhibited encouraging anticancer results. The order for the % control growth inhibition of

HepG-2 was found to be 8g<8i<8h<8j<8e<8b<8d<8c**(Table 2)**. Compounds (8a & 8f) inhibited 50% of the cell growth at the conc. <10 µg/ml. The compound 8f and 8g inhibited the total cell growth at the conc. <10 and 65.9 µg/ml respectivelyfor Andriamycin**(Table 3)**. The results pertaining to GI_{50} and LC_{50} have been presented in the Table 3and Figure 2.From the *in-vitro* analysis it has been found that compounds substituted with electron withdrawing group for *e.g.* NO_2, F, Cl, Br had better activity. The activity was directly proportional to the conc. of the compounds utilised to carry out the activity. The graphical representation showed that if the experiments were carried out at increased concentrations then the compounds would have shown better anti-cancer activity.

Conclusion

In conclusion, a total of ten 1,3,4-oxadiazolyl benzenesulfonyl benzimidazole derivative (8a-8j)**,** have been synthesized and evaluated for their anti-cancer activity. Among these synthesized heterocycles, compound 8a and 8fwere found to possess maximum anti-cancer activity. From the *in-vitro* analysis it has been found that compounds substituted with electron withdrawing group for *e.g.* NO_2, F, Cl, Br had better activity. The derivatives of 1,3,4-oxadiazolyl benzenesulphonyl benzimidazolecan be explored further for better anti-cancer activity by extensively studying the SAR (Structure Activity Relationship) as well as carrying out by fluorescent tubulin intensity assay method.

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Conflict of interest

The authors declare no conflict of interest.

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Table 1: Physical Characteristics of synthesized compounds (8a-j)

Comp. No.	Molecular formula	Colour	Melting point (°C)	R_f*	Yield (%)
8a	$C_{21}H_{13}N_5O_5S$	Off-white	251-252	0.56	77
8b	$C_{21}H_{13}ClN_4O_3S$	Light-brown	254-259	0.67	76
8c	$C_{21}H_{13}FN_4O_3S$	Beige	260-262	0.62	79
8d	$C_{21}H_{13}BrN_4O_3S$	Off-white	265-267	0.63	78
8e	$C_{21}H_{14}N_4O_4S$	Off-white	256-258	0.65	73
8f	$C_{22}H_{15}N_5O_5S$	Beige	240-242	0.52	75
8g	$C_{22}H_{15}ClN_4O_3S$	Beige	234-236	0.59	72
8h	$C_{22}H_{15}FN_4O_3S$	Off-white	221-223	0.57	69
8i	$C_{22}H_{15}BrN_4O_3S$	Reddish	251-254	0.61	70
8j	$C_{22}H_{16}N_4O_4S$	Brown	234-236	0.69	71

*Ethyl acetate: hexane (3: 1)

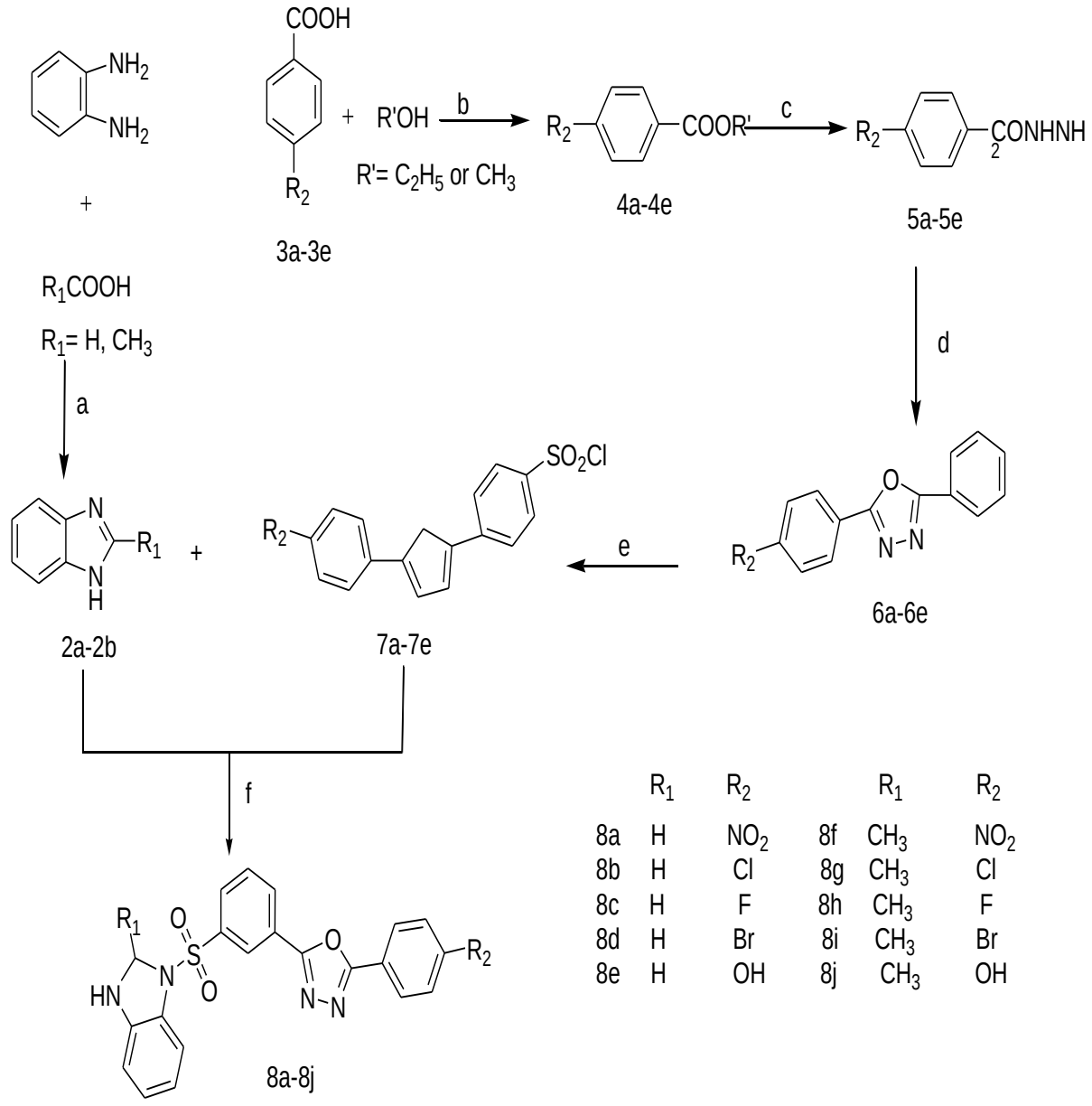
Table 2: *In vitro* percentage control growth of Human lung cancer cell line HepG-2 at different molar drug concentrations

C. No.	Drug Concentrations (µg/ml)															
	(Experiment 1)				(Experiment 2)				(Experiment 3)				Average % control growth			
	10	20	40	80	10	20	40	80	10	20	40	80	10	20	40	80
8a	24.2	43.7	43.8	38.4	20.8	22.1	25.7	31.6	28.1	25.1	22.0	27.2	24.4	30.3	30.5	32.4
8b	114.7	126.7	113.4	89.9	113.8	108.4	98.5	90.8	123.8	110.6	96.3	85.2	117.4	115.2	102.7	88.6
8c	117.7	120.8	95.5	49.8	113.8	102.0	86.5	54.7	113.9	109.5	88.8	52.3	115.1	110.8	90.3	52.3
8d	117.2	118.1	105.7	69.0	108.9	104.1	89.8	65.9	117.3	107.6	95.0	72.6	114.5	109.9	96.8	69.1
8e	125.7	123.2	124.5	96.9	116.8	112.4	104.3	100.5	129.8	119.7	116.7	103.3	124.1	118.4	115.2	100.3
8f	32.8	32.1	42.2	30.1	17.7	18.3	20.1	27.7	26.2	28.6	27.8	33.0	25.5	26.3	30.1	30.2
8g	116.0	103.2	76.6	74.0	115.2	99.5	80.4	74.6	124.2	106.2	88.1	81.0	118.4	103.0	81.7	76.6
8h	96.3	85.8	80.1	78.7	111.7	89.6	80.2	71.0	114.0	110.0	94.4	78.2	107.3	95.1	84.9	76.0
8i	99.4	92.7	82.3	62.4	109.5	95.7	79.4	64.0	117.2	116.6	99.7	74.2	108.7	101.7	87.1	66.9
8j	111.4	109.9	99.1	99.6	115.2	112.3	97.4	94.2	127.3	135.5	123.4	100.7	118.0	119.2	106.6	98.2
ADR	-35.2	-53.6	-61.4	-47.3	-39.1	-58.1	-65.4	-54.5	-29.7	-42.8	-56.1	-46.7	-34.7	-51.5	-61.0	-49.5

Table 3: TGI, LC_{50} and GI_{50} of the synthesized compounds against Human lung cancer cell line HepG-2

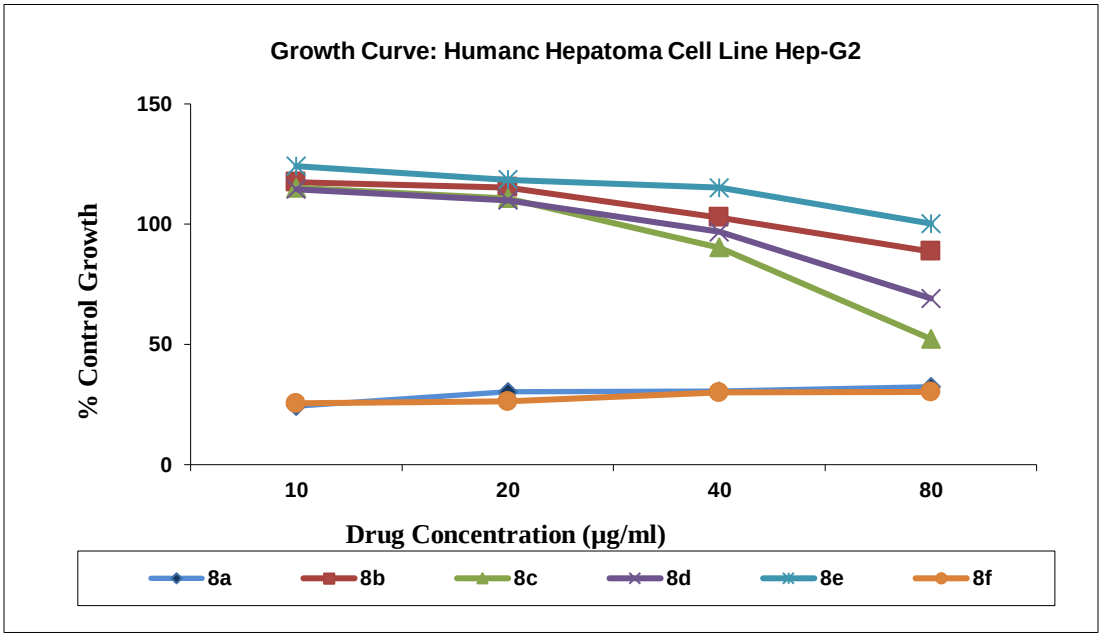
C. No.	LC_{50} (µg/ml)	TGI (µg/ml)	GI_{50}(µg/ml)
8a	NE	NE	<10
8b	NE	NE	>80
8c	NE	NE	>80
8d	NE	NE	>80

8e	NE	NE	>80
8f	NE	<10	<10
8g	NE	65.9	>80
8h	NE	NE	>80
8i	NE	NE	>80
8j	NE	NE	>80
ADR	43.56	<10	<10



Reagents and Conditions: a) heat at 100°C, 2hb) $NaHCO_3$, CCl_4, reflux ,4h c) hydrazine hydrate, ethanol, reflux, 3h d)C_6H_5COOH, $POCl_3$, reflux, 4he) chlorosulphonic acid, DCM, 50°C f) pyridine, DCM, rt, 2h.

Figure 1: Reaction scheme for the synthesis of 1,3,4-oxadiazolylbenzenesulfonylbenzimidazole derivatives



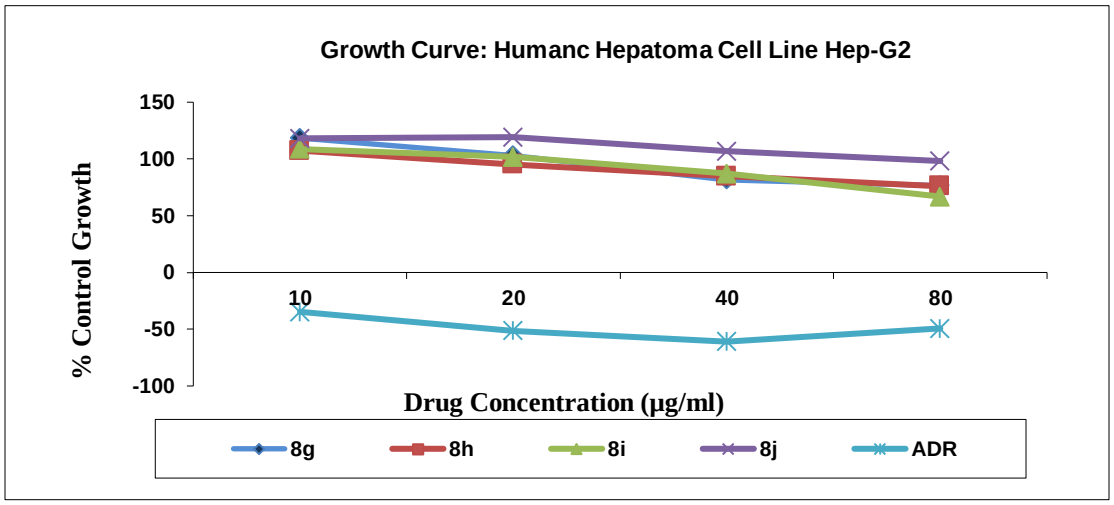


Figure 2: Graph showing % control growth against the drug concentrations (8a-8sj) and standard Andriamycin

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